

## **Assessment of Isoform specificity for a polyclonal Elastase ELISA**

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**Introduction:** Elastase is secreted from the pancreas and passes through the GI-tract without significant degradation. Exocrine pancreatic function analysis can therefore be performed with Elastase stool tests. Five different isoforms of human pancreatic elastase (PA I, IIA, IIB, IIIA, IIIB) have been identified. Three different polyclonal antisera that are used in a commercial ELISA were investigated for their specific recognition of human elastase isoforms in human pancreatic juice.

**Material and Methods:** Secreted proteins from human pancreatic juice were analysed by one or two-dimensional gel-electrophoresis, followed by westernblot analysis using 3 different polyclonal anti-Elastase antibodies (BIOSERV GmbH, Germany) and MALDI-TOF-MS. Elastase-activity was analysed in immunoprecipitates from human pancreatic juice using a fluorogenic Elastase substrate. Finally, cross-reactivity of the antibodies was tested against pancreatin from pig pancreas.

**Results:** In 1D Western blots of pancreatic juice all three polyclonal antisera against human Elastase detected a single ~30kDa protein. Immunoprecipitates with these antibodies exhibited elastase activity as determined with the fluorogenic Elastase substrate. In 2D-Westernblots (pH3-10) proteins in the molecular weight range of ~30 kDa were separated into a number of spots of different isoelectric points (pI). MALDI-TOF-MS-Analysis of these spots revealed the presence of pancreatic Elastase IIIA and IIIB isoforms, but not Elastase II or Elastase I isoforms. Western blot analysis of pancreatin from pig pancreas revealed no cross-reactivity with any of the three antisera tested.

**Conclusion:** All three commercial antibodies that are used in a polyclonal Elastase ELISA preferentially detect human Elastase isoforms IIA and IIIB, and do not cross-react with pig pancreatin. At present differences concerning expression and specific function of PA II or PA III isoforms are still unknown, but we could demonstrate, that PA III isoforms clearly possess Elastase activity as determined by a fluorogenic Elastase substrate. Elastase I is not an enzyme expressed in the adult human pancreas and should therefore not be referred to in commercial test kits for exocrine pancreatic function.

At present, differences concerning expression, secretion and specific function of pancreatic elastase 2 and 3 isoforms in humans are still unknown, and their specific prognostic value in the assessment of exocrine pancreatic insufficiency needs further evaluation.